Amplification of the MYCN oncogene on chromosome 2p24 is a strong prognostic factor for patients with neuroblastoma, regardless of age, staging, or other genetic aberrations. Few cases of neuroblastoma tumors with heterogenous patterns of MYCN amplification have been reported in the literature. The clinical significance of intratumoral MYCN heterogeneity is unclear. We present a case of an infant who presented with INSS stage 4S (or INRG stage 4S) neuroblastoma whose primary tumor in the adrenal gland contained a small nodule of MYCN-amplified cells surrounded by nonamplified tumor. This report highlights a dilemma for the clinician who must determine whether the patient qualifies for observation, or whether they have high-risk disease requiring aggressive chemotherapy.

CASE REPORT

A full-term male infant presented to his physician at 3 months of age with hepatomegaly. Before evaluation, he had been healthy, with appropriate feeding and weight gain for age. On physical examination, the liver was enlarged, firm and nodular on palpation, extending 5 cm below the right costal margin. Laboratory evaluation revealed the following: aspartate aminotransferase 144 IU/L; alanine aminotransferase 276 IU/L; prothrombin time 12.4 seconds (24.2 to 34.5 s); uric acid 5.2 mg/dL (1.8 to 5 mg/dL); activated partial thromboplastin time 37.9 seconds (24.2 to 34.5 s); urine homovanillic acid 53.7 mg/g UCr (ULN for age 22.5 mg/g UCr).

Ultrasound showed an echogenic liver with a nodular contour and several intrahepatic lesions. Computed tomography demonstrated a 1.9 × 2.0 × 0.8 cm hypodense mass arising from the left adrenal gland. The liver was enlarged and had a heterogenous appearance with a 6 mm hypodense lesion in segment 8. Magnetic resonance imaging showed the adrenal mass to be hyperintense on T2-weighted images and enhancing after contrast administration (Fig. 1A). The liver had a heterogenous appearance with multiple nodules that were hypointense on T2-weighted images and enhancing after contrast administration (Fig. 1B). On precontrast T1-weighted images, the liver was irregular with areas of decreased signal at the periphery. I-123-meta-iodobenzylguanidine scan showed increased uptake in the left adrenal mass (Fig. 1C) and along the periphery of the liver (Fig. 1D).

Bilateral bone marrow aspirates were performed, and the adrenal mass was resected. Aspirates demonstrated few small clusters of neuroblasts, <1% of the total marrow cells. The resected adrenal mass measured 1.8 × 1.5 × 0.8 cm. The cut surface was homogeneously dark red. Histologically, the tumor was composed of Schwannian stroma-poor, poorly differentiated neuroblasts. Mitotic karyorrhectic index (MKI) was low. However, within the mass there was a well-circumscribed 3-mm nodule (Fig. 2A) composed of Schwannian stroma-poor, poorly differentiated neuroblasts with a high MKI (Fig. 2B). DNA ploidy was 1.289; lp and 11q LOH studies were sent to the Children’s Oncology Group (COG) biology laboratory but were not performed because of unsatisfactory quality of the sample. Although fluorescent in situ hybridization (FISH) of the tumor submitted to the institutional pathology laboratory did not demonstrate MYCN amplification, samples submitted to the COG pathology reference laboratory showed MYCN amplification in 86% of cells in the high MKI nodule (Fig. 2C). The specimen was deemed a “composite neuroblastoma with unfavorable histology.”

Liver biopsy demonstrated an infiltrating nodule with fibrosis. The nodule was composed of nests of low MKI, CD56 positive, poorly differentiated tumor cells with crush artifact consistent with metastatic neuroblastoma (Fig. 2D). FISH of the liver slides did not reveal MYCN amplification at the institutional pathology laboratory, and was corroborated in the COG reference laboratory.

On the basis of the above findings, the patient was categorized as an INSS stage 4S neuroblastoma, and was followed with close observation. More than 24 months after diagnosis, the patient is doing well without recurrence. His most recent urine homovanillic acid and vanillylmandelic acid levels were 12.3 mg/g UCr (ULN 21.7 mg/g UCr) and 6.0 mg/g UCr (ULN 12.7 mg UCr), respectively. I-123MIBG shows no uptake in the adrenal surgical bed or at any distant site.

DISCUSSION

Amplification of MYCN is regarded as a high-risk feature and is well described in the literature. In the new INRG classification system, our patient would have been classified as Pretreatment Risk Group R (high risk), due to the presence of the MYCN-amplified nodule. Although there have been reports of good outcomes for patients with stage 4S disease and MYCN amplification...
without treatment, other studies have demonstrated survival rates ranging between 0% and 59%. Of those that reported treatment details, the use of adjuvant therapy did not seem to impact overall survival.

Few cases of MYCN heterogeneity in neuroblastoma have been reported and outcome data in these patients has been limited (Table 1). A 3-year-old girl with stage 3 neuroblastoma demonstrated both undifferentiated and differentiated areas, with MYCN amplification within the undifferentiated component. The patient did not respond to 3 cycles of cyclophosphamide with doxorubicin or teniposide. Despite intensification of chemotherapy with carboplatin and etoposide, she developed disseminated disease and died 7 months after diagnosis. Kerbl et al reported a patient with mixed patterns of MYCN and 1p36.3 imbalance who was in remission after cytotoxic therapy without

FIGURE 1. A, Axial T2-weighted image of the left upper abdomen shows a hyperintense mass (arrow) arising from the left adrenal gland. B, Axial T1-weighted image of the right upper abdomen shows a heterogenous appearance of the liver with peripheral areas of irregular hypointense signal. C, Anterior and posterior projections from an I-123 meta-iodobenzylguanidine (MIBG) scan shows a low level of uptake in the left adrenal mass (arrow). In addition to the adrenal mass, there is considerable uptake within the liver. D, Axial image from a I-123 MIBG SPECT/CT shows uptake of the radiotracer in the periphery of the liver in a similar distribution to the abnormality of the magnetic resonance imaging.
high-dose chemotherapy or autologous stem cell transplantation. Sano and colleagues described a child with stage 3 neuroblastoma who demonstrated 2 biologically distinct clones of favorable and unfavorable histology. The child received high-risk therapy and was in the sixth cycle of chemotherapy at the time of the report. Valent and colleagues reported 200 primary neuroblastoma tumors, 4 of which had variations of nonamplification, MYCN gain, and amplification between neighboring cells. One patient with stage 4S disease was observed, 1 died of treatment toxicity, and 2 were still receiving treatment at the time of publication. Theissen and colleagues recently conducted a review of patients from the German cooperative trials and identified 20 patients whose tumors demonstrated heterogeneous MYCN amplification. Twelve subjects had clusters of MYCN-amplified cells within their primary tumor at diagnosis and 2 subjects had single-cell amplifications. Five patients with localized or 4S disease and MYCN-amplified cells received no chemotherapy; 4 remain disease free at follow-up ranging from 1 to 11.5 years.

Limited clinical information is available to develop a treatment algorithm for patients with localized or 4S disease.
disease whose risk status may be elevated by the presence of heterogenous MYCN amplification. Although 4 of the 5 of these patients in the German cohort had good outcomes without chemotherapy, the rarity of intratumoral MYCN heterogeneity likely prevents detailed analysis of this subset. In our patient, the liver biopsy was felt to be informative in that the pathology supported a metastatic process driven by the predominant, nonamplified neuroblastoma, rather than aggressive MYCN-amplified disease. However, a lack of data in the literature on the impact of MYCN heterogeneity in metastatic lesions on survival also clouds clinical judgment. Of the cases of heterogenous tumors with stage 4 or 4S disease identified, few report the presence or absence of MYCN amplification in metastatic lesions, as sampling of metastatic deposits is not typically performed. Those who include metastatic evidence are limited to marrow disease, which is not unexpected given the role of bone marrow evaluation in standard diagnostic evaluation. Outcomes are mixed for these subjects; a patient with focal amplification within the primary tumor and nonamplified bone marrow disease is alive after 6 cycles of chemotherapy. Another patient with amplification in both primary tumor and bone marrow died of disease, and a third with nonamplified primary tumor but a MYCN-amplified cluster of cells in the bone marrow relapsed after 14 months and died 20 months after diagnosis.

Findings of MYCN heterogeneity within a tumor raise questions regarding the nature of MYCN as a primary or secondary feature of neuroblastoma. Studies of TH-MYCN transgenic mouse models have shown that inappropriate perinatal MYCN expression prevents regression of paravertebral ganglion neuroblast hyperplasia, and further changes such as MYCN amplification are associated with tumor progression in hemizygous mice. The hyperplastic lesions are composed of Phox2B + neuronal progenitors, and MYCN promotes their proliferation while simultaneously preventing their differentiation. High levels of Phox2B mRNA expression have been identified in primary neuroblastoma tumors, and Phox2B expression has been demonstrated in xenografts derived from human neuroblastoma cells, independent of the status of MYCN amplification. These findings may suggest that MYCN amplification plays a secondary role in neuroblastoma tumorigenesis by driving previously established Phox2B +

### TABLE 1. Examples of MYCN Heterogeneity in Neuroblastoma From Prior Publications

<table>
<thead>
<tr>
<th>References</th>
<th>No. Samples</th>
<th>MYCN Heterogeneity At Diagnosis</th>
<th>Method of MYCN Detection</th>
<th>Staging (INSS)</th>
<th>Treatment</th>
<th>Outcome</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lorenzana et al 12</td>
<td>1</td>
<td>FISH, Southern blot</td>
<td></td>
<td>3</td>
<td>Chemotherapy</td>
<td>Died of disease</td>
<td></td>
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<tr>
<td>Ambros et al 13</td>
<td>300</td>
<td>FISH, Southern blot</td>
<td>Localized (2) 4S (1)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Heterogeneity of 1p36.3 deletions demonstrated in 3 additional tumors</td>
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<tr>
<td>Kerbl et al 14</td>
<td>1</td>
<td>FISH</td>
<td></td>
<td>4S</td>
<td>Chemotherapy x 6 cycles NA</td>
<td>NED 13 mo</td>
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<tr>
<td>Spitz et al 15</td>
<td>659</td>
<td>FISH, Quantitative RT-PCR</td>
<td></td>
<td>NA</td>
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<td>Valenti et al 16</td>
<td>200</td>
<td>FISH</td>
<td></td>
<td>3 (1)</td>
<td>Chemotherapy</td>
<td>Alive, in treatment</td>
<td>Died of toxicity</td>
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<td></td>
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<td></td>
<td>4 (1)</td>
<td>Chemotherapy</td>
<td>Alive, in treatment</td>
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<td></td>
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<td>4S (2)</td>
<td>Observation (1) NA</td>
<td>NED 13 mo</td>
<td>NA</td>
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<td>Thorner et al 17</td>
<td>41</td>
<td>Chromogenic in situ hybridization (CISH), FISH, Southern blot, PCR</td>
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<td>NA</td>
<td>Observation (1) NA</td>
<td>Alive, in treatment</td>
<td>NA</td>
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<tr>
<td>Sano et al 18</td>
<td>1</td>
<td>FISH</td>
<td></td>
<td>3</td>
<td>Chemotherapy</td>
<td>Alive, in treatment</td>
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<tr>
<td>Canete et al 11</td>
<td>46</td>
<td>FISH</td>
<td></td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Excluded from analysis because of MYCN heterogeneity</td>
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<tr>
<td>Theissen et al 19</td>
<td>1341</td>
<td>FISH</td>
<td></td>
<td>1 (2)</td>
<td>High risk (1) Observation (1) NED 59 mo</td>
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<td>3 (5)</td>
<td>High risk (3) NED (2) Local progression (1)</td>
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<td>4 (6)</td>
<td>High risk (6) NED (1), Local progression (1)</td>
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<td>4S (2)</td>
<td>Observation (2) NED (1), Died of disease (5)</td>
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FISH indicates fluorescence in situ hybridization; INSS, International Neuroblastoma Staging System; NA, not available; NED, no evidence of disease; PCR, polymerase chain reaction; RT-PCR, reverse transcriptase polymerase chain reaction; SCT, stem cell transplantation.
neuronal progenitors toward an aggressive clinical phenotype with poorer prognosis. Additional information is needed regarding other biological markers that may help guide the clinician in determining appropriate therapy. In the INRG cohort, 11q loss of heterozygosity was identified as the most important prognostic factor in patients with MYCN-nonamplified stage 4S tumors. However, the small number of 4S patients in the cohort requires further evaluation to determine its true impact. DNA ploidy > 1.0 carried prognostic significance for children younger than 18 months with stage 4, MYCN-nonamplified neuroblastoma (EFS 85% vs. 71%), but its impact on 4S tumors is not as well characterized in the literature. In addition, a review of 87 low-stage tumors with MYCN amplification showed better outcomes for patients with hyperdiploid tumors than diploid tumors. Positive findings for genetic characteristics such as 11q aberrations may suggest the presence of higher risk disease that may require aggressive chemotherapy. Conversely, identification of hyperdiploid cells within a heterogenous sample might suggest less aggressive disease amenable to treatment reduction. The decision to observe our patient was based on the fact that the MYCN-amplified focus was small and limited to the primary adrenal tumor, and that both the liver and bone marrow involvement had biologically favorable characteristics consistent with conventional stage 4S disease. Unfortunately, full DNA analysis for 11q and 1p LOH studies could not be completed on the available tumor specimen. Given the presence of a small nodule of MYCN-amplified tissue, the possibility of late relapse exists, but previous reports have identified patients with similar characteristics who were observed and remain disease free up to 11.5 years after diagnosis. Although a clear pathway is not available for these rare patients, our patient remains free of disease for >24 months from diagnosis without chemotherapy, and continues under close observation.

ACKNOWLEDGMENT

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REFERENCES